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Development of oogonia of *Sargassum horneri* (Fucales, Heterokontophyta) and concomitant variations in PSII photosynthetic activities

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Abstract: *Sargassum horneri* is one of the most important seaweeds used to restore degraded coastal environments near Nanji Island, China. However, its reproductive characteristics, especially those of oogenesis and the concomitant changes in photosynthetic activities, remained uncertain. Herein we documented the processes of conceptacle and female gamete formation and the photosystem II (PSII) changes that accompanied them. Thalli of *S. horneri* were dioecious, the oogonial conceptacles maturing acropetally on the axillary receptacles. Conceptacles were originated from the epidermal cells, the cells lining the inner surface of the cavity ultimately differentiating into either sterile unbranched paraphyses or oogonia. The PSII photosynthetic activities of oogonia continuously diminished with increasing maturation. The value of Fv/Fm decreased from 0.55 ± 0.01 for recently initiated oogonia to 0.27 ± 0.05 for mature oogonia. The diameters of the oogonia increased approximately 20 times from initiation to maturity. The possible nutrition sources of oogonia during their development are discussed.

Key Words: Fucales, Morphogenisis, Oogenesis, PS II, *Sargassum horneri*

INTRODUCTION

Sargassum horneri (Turner) C. Agardh is one of the dominant seaweed species along the coast of Nanji Island, Zhejiang Province, China. The thalli of *S. horneri* reach 7–8 m in length and, together with other *Sargassum* species, form a *Sargassum* forest. The *Sargassum* forest plays important ecological roles by providing habitat for many marine animals and plants and by absorbing large amounts of nitrogen and phosphorous (Komatsu *et al*., 1990; Fei 2004). However, coastal highway construction and other anthropogenic activities caused a decrease in water transparency and the loss of natural substrata that is required for germling adhesion (Sun *et al*. 2008a). Consequently, *S. horneri* populations have been decreasing at an extremely rapid pace. Cultivation of *S. horneri* seedlings, followed by transplantation to natural habitats, effectively restored degenerated beds in Japan and China (Yamauchi 1984; Choi *et al*. 2003; Terawaki *et al*. 2003). Sun *et al*. (2008b) reported that the population density of *S. horneri* at Nanji Island was sustained principally through sexual reproduction; by contrast, the vegetative reproduction from the holdfast only represented 5%. Therefore, a comprehensive understanding of the reproductive characteristics, particularly the formation of sexual reproductive organs and gametes, is important for understanding how populations can best be maintained and propagated.

The reproductive properties in members of *Sargassum* have been reported in numerous studies (Vijayaraghavan & Kaur 1991; Kaur & Vijayaraghavan 1993, 1994). One of the striking characteristics is the expulsion of oogonia from conceptacles. Eggs within a given conceptacle of *S. horneri* are not expelled simultaneously from the receptacles; instead, eggs within the more proximal conceptacles are released first, followed progressively by more distal conceptacles (Kaur & Vijayaraghavan 1992; Pang *et al*. 2009). Although the photosynthetic activities of eggs and embryos of *Fucus* spp. have been comprehensively investigated (McLachlan & Bidwell 1978; Amsler & Neushul 1991; Lamote *et al*. 2003), little is known of these phenomena in *S. horneri*.

In this study we trace the dynamic changes in the morphological and photosynthetic characteristics of eggs during their development within given receptacles using serial paraffin sections and Imaging-Pulse Amplitude Modulation fluorometry. Our results suggest that by adjusting light intensities we can improve the efficiency for indoor-reared germlings.

MATERIAL AND METHODS

Female thalli of *S. horneri* were collected from the subtidal zone at Nanji Island, Zhejiang Province (27°27′N, 121°05′E), China on 26 April 2010. Fronds were cultured in the laboratory without agitation in a tank containing *c*. 30 litres of sterile seawater at maintained at 20°C. The receptacles were examined daily for release of gametes. Approximately 2 d after collection when collection was near the full moon, large amounts of oogonia were released; the release of oogonia in the laboratory tanks was not closely dependent on the tidal cycle. When egg release was first detected in the proximal portions, those receptacles were immediately

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removed from the tank and divided into two groups; one group was used for photosystem II (PSII) photosynthetic activity measurements and the other group was used for serial paraffin sections. Both groups consisted of at least four receptacles.

Imaging-PAM with Maxi-head (Walz GmbH, Effeltrich, Germany) was used to measure the PSII photosynthetic activities of released and unreleased areas of the receptacles. Samples were dark-adapted for 15 minutes and then used for determination of variable fluorescence/maximal fluorescence (Fv/Fm). Under very weak light, the minimal fluorescence (Fo) was obtained and Fm then induced by saturated pulse. Maximal PSII quantum yield (Fv/Fm) was calculated according to following formula: $Fv/Fm = (Fm - Fo)/Fm$ (Roháček *et al*. 2008). Receptacles from which some eggs had been released were cut into 0.5–1-mm thicknesses to compare the PSII photosynthetic activities of epidermal cells and oogonia at different positions along the same receptacle using Microscopy-PAM (Walz GmbH, Effeltrich, Germany). Sample treatment and the measurement method were the same as those used for Maxi-PAM. Before measurement, cross-sections were examined to ensure that only undamaged oogonia were being measured for photosynthetic activity. Two replicate experiments were performed.

The protocols for the preparation of serial paraffin sections were modified from Xie *et al*. (2010). Receptacles were transversely cut into small fragments of about 0. 5 cm in length, which were then fixed for 48 hours in a solution of 70% ethanol (90 ml), 100% acetic acid (5 ml), and 40% formaldehyde (5 ml); the fixing solution was replaced at 8-h intervals. Receptacles were then dehydrated in graded ethanol solutions of 70% (2.5 h), 83% (2 h), 95% (2 h), and 100% (1 h). Before embedding in paraffin, conceptacles were treated in 100% ethanol:xylene (1:1, 2 h), followed by 100% xylene (1 h). Samples were kept at 37°C until embedded in paraffin. The paraffin-embedded receptacles were sliced at a thickness of 10 μm with a Leica 2016 microtome (Leica Instruments Ltd., Shanghai, China). Sections were rehydrated for microscopic examination in the following sequence: 100% xylene (10 min), 100% ethanol (2 min), 95%, 80%, 75% ethanol solutions (1 min each), and distilled H_2O (2 min). Sections were stained with hematoxylin–eosin. A Nikon 90i microscope (Nikon Corp., Tokyo, Japan) was used to observe and photograph the sections.

Data shown in Fig. 19 were means of the values for more than three oogonia. Statistical significance was tested by one-way ANOVA using SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA). The graphs were plotted with OriginPro 8.0 (OriginLab Corporation, Northampton, Massachusetts, USA).

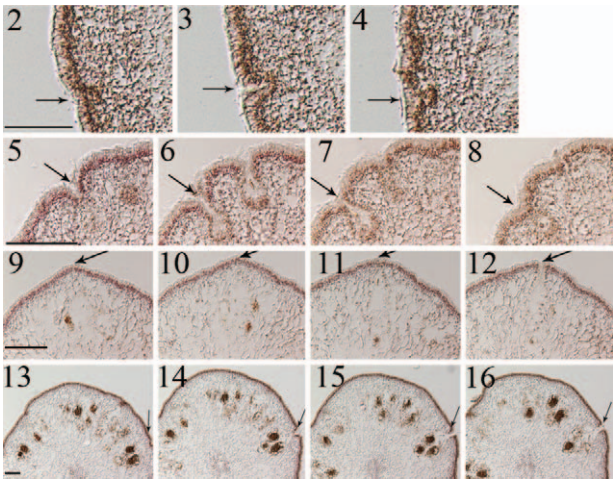
RESULTS

Receptacles in *S. horneri* were unusual in the genus *Sargassum* because they were simple (Fig. 1) rather than compound. Mature receptacles reached about 2 cm in length and released large numbers of eggs, which largely remained on the surface where they were fertilized by sperm released from antheridia that were formed inside conceptacles of male gametophytes. The more proximal conceptacles always released eggs 2–3 d earlier than the distal ones. The acropetal maturation of conceptacles and eggs was verified from progressively more distal cross-sections (Figs 1A–D, 2–16). Conceptacles were initiated near the receptacle apex where the diameter was 398.61 ± 43.75 μm and the conceptacle depth reached 60.21 ± 4.17 μm (Figs 2–4). The cells that lined the floor of the conceptacle were morphologically similar and undoubtedly homologous to the epidermal cells from which they were derived. No oogonial primordia were yet present. When the receptacle diameter increased to 502.88 ± 53.64 μm (Figs 5–8), the depth of the conceptacle increased to 95.96 ± 7.55 μm. Although the diameter of the ostiole was only 17.03 ±1.99 μm, the diameter of the chamber base reached 51.55 ± 0.92 μm (including the bottom floor cells) and the overall shape of the conceptacle became lacrimose. Proximally, the conceptacles reached 203.67 ± 8.08 μm in depth and their inner-surface cells, most of them 10.25 ± 2 in diameter, began to divide toward the lumen of the conceptacle and formed unbranched paraphyses (Figs 9–12). Only a few (four) of the liner cells had differentiated into oogonial primordia with a diameter of 28.6 ± 8.62 μm. The most proximal conceptacles where oogonia had not been expelled had egg diameters of 102.8 ± 7.95 μm (Figs 13–16), i.e. 3.6 times greater than those at mid-development (Figs 9–12) and 4.5 times greater than the diameter of the precursor epidermal cells (22.78 ± 0.80 μm diam.).

Receptacles in which conceptacles of the proximal half had released oogonia were used to determine the PSII maximal quantum yield (Fv/Fm) of oogonia of different maturities (Figs 17–31). The values of Fv/Fm for released and unreleased areas within semireleased receptacles differed significantly ($n = 7$, $P < 0.05$); the value for unreleased areas was about 0.632 ± 0.04, whereas that for released areas was about 0.481 ± 0.07 (Figs. 17, 18).

Sectioned receptacles were examined to further determine the maximal PSII quantum yields of oogonia at different stages of maturity and to compare the numbers with those for epidermal cells. All epidermal cells, regardless of location, possessed equivalent Fv/Fm values (0.61 ± 0.01; Fig. 19, line of solid black circles) and no statistical difference was detected between them ($P > 0.05$). In contrast, the values of Fv/Fm for oogonia at different developmental stages exhibited marked differences (Fig. 19, line of open circles). For young oogonia with a diameter of 25.98 ± 5.12 μm, the value of Fv/Fm was 0.55 ± 0.01, which was lower than that of epidermis cells (Figs 20, 26). When oogonia reached 132.11 ± 25.05 μm in diameter, the PSII maximal quantum yield decreased to 0.43 ± 0.03 (Figs 21, 27). For mature oogonia with diameters of 225.12 ± 19.77 μm, 272.31 ± 31.33 μm, and 260.78 ± 55.16 μm (Figs 22, 28, 23, 29, 24, 30) but which were still not expelled from proximal conceptacles, the values of Fv/Fm were 0.25 ± 0.02, 0.30 ± 0.05, and 0.26 ± 0.04, respectively. Released eggs that were retained on the surface of the receptacle had a Fv/Fm value of 0.25 ± 0.06 (Figs 25, 31), which was virtually identical to the values of mature unreleased oogonia. Statistical analysis revealed that there was no significant difference among the values of Fv/Fm for these mature oogonia and released eggs, but a significant difference did exist between the epidermis cells, the immature

Fig. 1. A female receptacle of *S. horneri* in which eggs released from conceptacles in the proximal half adhere through mucilage to the receptacle surface. The distal points A–D indicate the positions of the cross-sections illustrated in Figs 2–16**.** Scale bar = 1 cm.



Figs 2–16. Progressively maturing conceptacles from initiation (Figs 2–4) to the maturity of oogonia (Figs 13–16). Scale bars = 100 μm.
Figs 2–4. Serial sections of oogonial initiation (arrows) taken at position A in Fig. 1.
Figs 5–8. Serial sections of oogonia (arrows) taken at position B in Fig. 1.
Figs 9–12. Serial sections of oogonia (arrows) taken at position C in Fig. 1.
Figs 13–16. Serial sections of oogonia (arrows) taken at position D in Fig. 1.

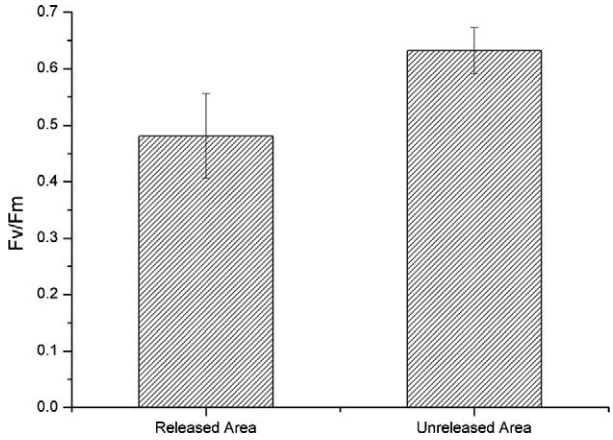


Fig. 17. Comparison of *F*v/*F*m values between proximal and distal oogonial receptacles.

oogonia, and the mature oogonia ($P < 0.05$). On the basis of the paraffin sections, we summarised the development process of the oogonia (Fig. 32).

DISCUSSION

Before this study, little was known about the PSII photosynthetic activities of unfertilized eggs in *S. horneri*, although much is known of these phenomena in other members of the Fucales. Lamote *et al.* (2003) investigated the changes in photosynthetic pigment composition and PSII photosynthetic activities of the fertilized eggs of *Fucus serratus* Linnaeus during the six days after fertilization. They found that variable fluorescence could not be detected until 30 h after fertilization, and the total amounts of photosynthetic pigments (mainly chlorophyll *a* and fucoxanthin) differed greatly between eggs and embryos. Comparison of the PSII photosynthetic activities between unfertilized eggs and embryos of *Ascophyllum nodosum* (Linnaeus) Le Jolis was conducted by Kim *et al.* (2006); they found that the maximal PSII quantum yield of newly released eggs was as high as that of embryos. In our study, we detected distinct changes in PSII photosynthetic activities of *S. horneri* throughout the process of oogenesis, from the initiation of oogonia to their maturity. These results are similar to those of Lamote *et al.* (2003) than are those of Kim *et al.* (2006), although there are some discrepancies. According to Lamote *el al.* (2003), variable fluorescence was absent in the zygotes of *F. serratus* for the first 30 h, implying that the unfertilized eggs would not possess variable fluorescence. In contrast, we found that although the value of *F*v/*F*m decreased during the development of *S. horneri* oogonia, fluorescence varied between mature oogonia and released eggs. This difference in results may be due to the different outcomes reached by oogonial development in *S. horneri* and *F. serratus*. In *F. serratus*, oogenesis results in eight eggs in each oogonium, whereas in *S. horneri*, oogonia undergo three karyokineses but no cytokinesis, resulting in single eggs with one functional and seven supernumerary haploid nuclei (Critchley *et al.* 1991; Lee 2008). Why this

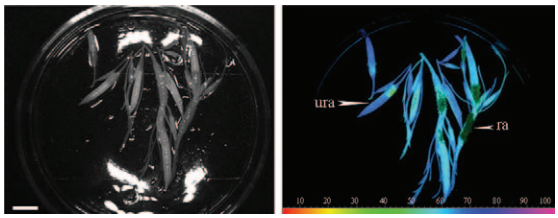


Fig. 18. Black-and-white image of receptacles (left) and corresponding false-colour image of *Fv*/*Fm* (right, the bottom colour bar indicating the relative value of *Fv*/*Fm* as a percentage). ra = released-oogonial area; ura = unreleased-oogonial area. Scale bar = 1 cm.

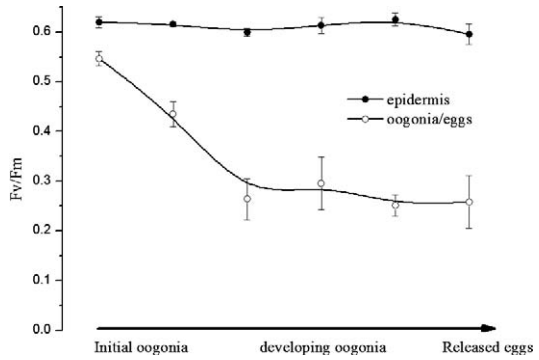
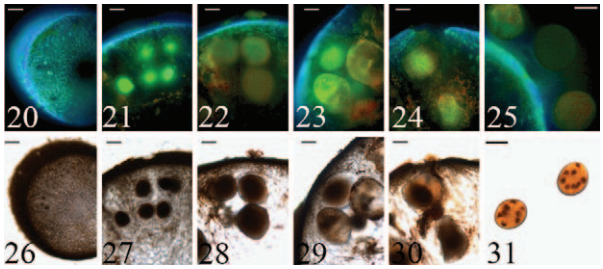


Fig. 19. Variation in the value of *Fv*/*Fm* for epidermal cells (solid circles) and oogonia (open circles) at different positions along a female receptacle.



Figs 20–25. False colour variation in the value of *Fv*/*Fm* for epidermal cells and oogonia at different positions along a female receptacle with accompanying false color (compare with Fig. 19). Scale bars = 100 μm.

Figs 26–31. Corresponding light microscopic images for each cross-sectional measurement (compare with Figs 20–25). Released eggs, which were attached on the surface of the receptacle (see Fig. 25), were detached from the surface as shown in Fig. 31. Scale bars = 100 μm.

difference should affect fluorescence phenomena, however, requires more study.

Our results showed that the diameters of *S. horneri* oogonia increased approximately 20 times from those of the precursor inner-lining cells (Figs 2–16, 26–31). Meanwhile, the value of *Fv*/*Fm* continuously decreased with increasing maturation of the oogonia (Figs 19–25), which means that the efficiency of PSII-dependent linear electron flow must be decreasing. It is not unlikely that this lowered efficiency would negatively influence the production of the reducing equivalent and further the photosynthetic CO_2 assimilation. It thus seems reasonable to assume that the material required for the growth and development of the oogonia is not produced by the oogonia themselves but is most likely transported from other tissues through some sort of conducting system. Similar to the members of the Laminariales, which have evolved sieve elements for the translocation of photosynthetic products such as mannitol (Parker 1965; Sideman & Scheirer 1977; Raven 2003), sieve elements have also evolved in members of the Fucales. The Fucales sieve elements also function as conducting tissues for translocation of photosynthetic products to younger apical regions, although the rate of transport is very low (Moss 1983; Diouris & Floc'h 1984; Raven 2003). In the case of receptacles of *Sargassum*, medullary cells are longitudinally connected through the sieve plate (Hales & Fletcher 1992) and the other tissues (e.g. epidermis, cortex, medulla) are also connected through pit junctions (Speransky *et al*. 2001). Furthermore, the cytoplasm of the oogonium and the conceptacle floor cells are connected by plasmodesmata or pit junctions (McCully 1968; May and Clayton 1991). Through these conducting systems, it is possible that movement of material from tissues with high PSII photosynthetic activities to the oogonia may be taking place, in which case the epidermal cells of receptacles or other vegetative photosynthetic tissues whose PSII photosynthetic activities are very close in other fucoid algae (Kim & Garbary, 2009) are the most likely source. However, further investigation on the translocation of ^{14}C-labelled photoassimilates may help to elucidate nutrition transport.

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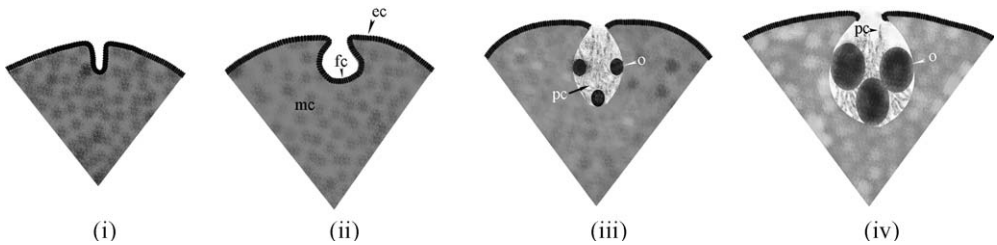

Fig. 32. Schematic representation of conceptacle developmental stages from initiation of oogonia (i) to maturity of oogonia (iv). ec = epidermal cell; fc = floor cell of conceptacle; mc = medullary cell; o = oogonium; pc = paraphysis cells.

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